# THE ULTRASTRUCTURE OF SPERMATOZOA OF *NANGURA SPINOSA* (SCINCIDAE, REPTILIA)

B.G.M. JAMIESON AND D.M. SCHELTINGA

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Spermatozoa of *Nangura spinosa* are filiform, and approximately 85 µm long. The acrosome vesicle in the form of an elongate hollow, concentrically zoned cone is compressed near its tip, and basally overlies a subacrosomal cone. Axial within the acrosome vesicle is a slender rod, the putative perforatorium. The subacrosomal cone is paracrystalline and invests the tapered anterior end of the nucleus. The perforatorium is a slender, slightly oblique rod extending anteriorly from the subacrosomal material. A conspicuous laminated structure forming a wing-like projection on each side of the proximal centriole contacts with the first of the dense 'ring structures' of the midpiece. The midpiece contains four dense ring structures in longitudinal succession, posterior to which lies the much smaller annulus, all being separated by mitochondrial regions. The mitochondria mostly form 12 or more elongate, sinuous columnar structures, with numerous predominantly longitudinal cristae. Nine peripheral dense fibres are associated with the 9 triplets of the distal centriole and the doublets of the axoneme. However, within the midpiece, only those peripheral fibres adjacent to doublets 3 and 8 remain conspicuous to the level of the annulus, each as a double structure associated with the annulated fibrous sheath. All peripheral fibres are absent from the principal piece. Similarity to the sperm of *Ctenotus* supports placement of *Nangura* in the *Sphenomorphus* group. Comparisons with other amniote sperm are made. □ *Nangura spinosa, Scincidae, spermatozoon, ultrastructure, phylogeny.*

*B.G.M. Jamieson and D.M. Scheltinga, Department of Zoology, University of Queensland, Queensland 4072, Australia; 14 October, 1993.*

Published descriptions of the male gametes of the Scincidae are limited to a description of the mature spermatozoon of *Chalcides ocellatus tiligugu* by Furieri (1970); an account of spermiogenesis, with some description of mature, epididymal sperm, in the same subspecies (Carcupino et al., 1989); and a very brief account of the development of the midpiece in *Eumeces laticeps* by Okia (1990). A description of the spermatozoon of the newly described genus and species, *Nangura spinosa* (see Covacevich et al., 1993), allows the addition of morphological characters from the spermatozoon to the description of this species and extends knowledge of skink spermatozoa. Comparison with the sperm of other reptiles will be limited chiefly to that necessary to determine which characters or character states appear to be, from the small sample, distinctive of the Scincidae and of this species. Although the Reptilia is an invalid, paraphyletic grouping (e.g. Jamieson & Healy, 1992), the term 'reptile' is here retained for convenience.

The ultrastructure of spermatozoa or spermiogenesis has been studied in the major groups of squamate reptiles, in addition to skinks: Lacertidae - Butler & Gabri (1984), Courtens & Depeiges (1985), Furieri (1970); Agamidae - Al-Hajj et al. (1987), Dehlawi et al. (1992), Charnier et al. (1967); Chameleonidae - Tuzet & Bourgat (1973); Iguanidae - Furieri (1974), Saita et al. (1988a); Anolidae - Clark (1967); Gekkonidae - Furieri (1970), Phillips & Asa (1993); Teiidae - Del Conte (1976), Newton & Trauth (1990, 1992); Tropiduridae - Da Cruz-Landim & Da Cruz-Höfling (1977), Furieri (1974); and Serpentes - Austin (1965), Boissin & Mattei (1965, 1966), Furieri (1970), Hamilton & Faweett (1968), Saita et al. (1988b), Phillips & Asa (1993).

Non-squamate reptiles which have been investigated are: Chelonia - De et al. (1987), Furieri (1970), Hess et al. (1991), Sprando et al. (1988), Yasuzumi & Yasuda (1968), Yasuzumi et al. (1971); Sphenodontida - Healy & Jamieson (1992), Jamieson & Healy (1992); and Crocodilia - Saita et al. (1987).

## MATERIAL AND METHODS

Small pieces of testis were taken from a single specimen of *Nangura spinosa* Covacevich, Couper and James, 1993, collected from Nangur State Forest, near Murgon, SEQ. These samples

were diced into 1-2mm$^3$ portions, and fixed for transmission electron microscopy (TEM), in 3% glutaraldehyde in 0.1M sodium phosphate buffer (pH 7.2), at 4°C for 2 hours and agitated for the first hour. The material was then rinsed in 0.1M phosphate buffer; post-fixed for 80 min in similarly buffered 1% osmium tetroxide; rinsed in buffer; dehydrated through an ascending ethanol series; and infiltrated and embedded in Spurr's epoxy resin. Sections were cut with diamond knives, on an LKB 2128 UM 1V microtome. Thin sections, 50-80 nm thick, were collected on carbon stabilized, colloidin-coated, 200 µm mesh copper grids, rinsed in distilled water, stained for 30 s in Reynold's lead citrate, then in 6% aqueous uranyl acetate for 4 min and for a further 2 min in lead citrate before final rinsing. Electron micrographs were taken on an Hitachi 300 electron microscope at 75 kV and a JEOL 100-s electron microscope at 60 kV. Light microscopic observations of spermatozoa, from glutaraldehyde-fixed tissue squashes, were made under Nomarski contrast using an Olympus BH2 microscope.

## RESULTS

Spermatozoa of *Nangura spinosa* (Fig. 1) are filiform, and approximately 85 µm long (mean of 10 = 85.4 µm, S.D = 2.8). A cytoplasmic droplet seen in some sperm, by light microscopy and by transmission electron microscopy, is located immediately behind or slightly overlapping the base of the nucleus. Dimensions (for one or two sperm) are: 5.5 µm for the length of the acrosome complex; 6.6 µm for the nucleus posterior to the acrosome;7.6 µm for the midpiece, from transmission electron microscopy, and, from light microscopy 66 µm for the flagellum behind the midpiece (principal piece). The head (acrosome and nucleus), and often the midpiece and flagellum, is curved (Fig. 2A). As a result of this curvature it has not been possible to obtain a complete longitudinal section through the head. The sperm is circular in cross section with the exception of the acrosome. Although the acrosome is circular at its base, anterior to this it develops a unilateral ridge and anterior to the tip of the subacrosomal cone it becomes increasingly compressed and elliptical in transverse section (Figs 2D-I).

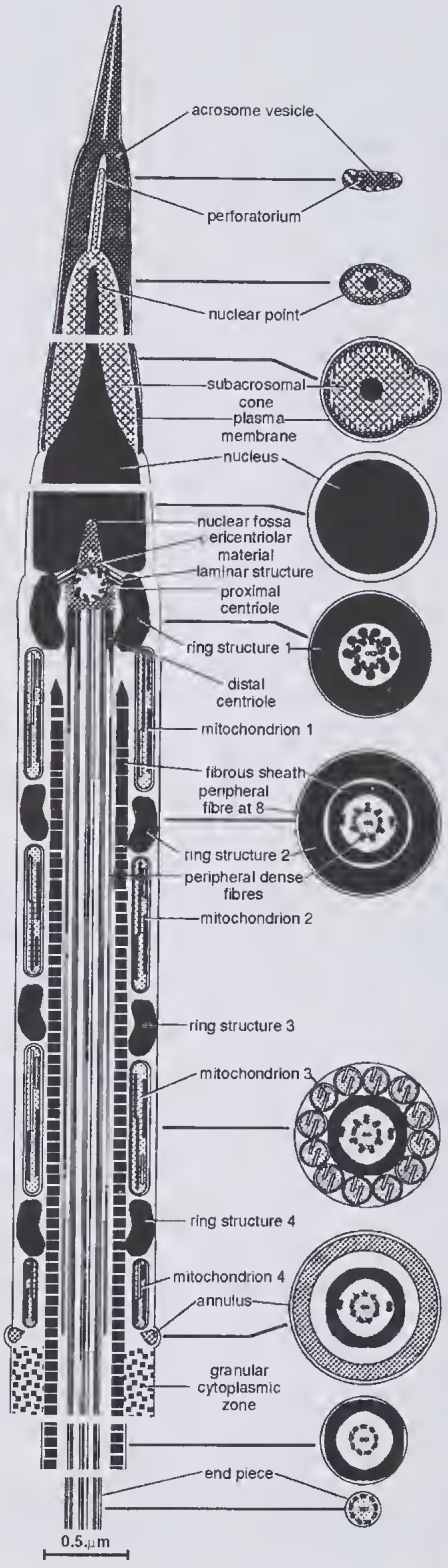



FIG. 1 *Nangura spinosa*. A diagrammatic summary of the spermatozoon viewed in longitudinal section, with the corresponding transverse sections.

### Acrosome Complex

The acrosome complex consists of an acrosome vesicle in the form of an elongate hollow cone, an underlying subacrosomal cone and, axial within the acrosome vesicle, a slender rod, the putative perforatorium. The acrosome complex is 5.5 μm long (Fig. 4A). The anterior end of the vesicle, comprising slightly less than half its total length, forms a thick walled hollow cone with a narrow lumen housing the perforatorium (Fig. 2B). The longer, posterior region of the vesicle is a thin walled continuation of this hollow cone, the vesicle here being no more than a sleeve-like investment (acrosome sleeve) of the subacrosomal material, as seen in longitudinal (Figs 2B, K, 4A) or transverse section (Figs 2F-I). The underlying subacrosomal material forms a thick relatively pale layer.

The material of the subacrosomal cone is paracrystalline, its matrix having fine obliquely longitudinal and less distinct transverse striations, indicating that it forms a fine lattice (Fig. 2C). For most of its length, from its posterior end anteriorly, the subacrosomal cone invests the tapered anterior end of the nucleus (nuclear point). Although corresponding with the subacrosomal cone of other amniote and amphibian sperm, it is not strictly conical but consists of material with a poorly defined outer border which fills the posterior space within the acrosomal sleeve (Figs 2B, C, F-H, K). The nuclear point terminates within the anterior limit of the subacrosomal material at an epinuclear electron lucent region (Fig. 2K).

The perforatorium is a slender, moderately electron dense rod, with some signs of internal longitudinal fibres. It extends anteriorly from the subacrosomal material, lying in a narrow lumen internal to the inner acrosome membrane (Figs 2B, D, 4A). It has been observed to extend through approximately the posterior half of the thick walled part of the acrosomal vesicle. Whether or not a pale, central axial tube-like structure which extends to the tip of the acrosome and displays some internal longitudinal fibres is a forward continuation of the perforatorium has not been determined (Fig. 2B). The perforatorium makes contact at its posterior end with the subacrosomal material. Even allowing for the pronounced curvature of the acrosome, the longitudinal axis of the perforatorium appears to be slightly oblique relative to that of the acrosome vesicle (Figs 2B, D, 4A).

In transverse sections of the acrosome vesicle through the nuclear point and perforatorium (Figs 2D-I), the vesicle is seen to have a concentric zonation which in sequence from the perforatorium outwards is: a narrow space around the perforatorium; a wide, dense, homogeneous zone; a narrow zone with radial striations; a thin, dense, homogeneous layer apposed to the plasma membrane.

### Nucleus

The nucleus is curved and tapers to a point within the basal region of the acrosome (acrosome sleeve). The transition from the tapered region (nuclear point) to the much longer cylindroid region is abrupt but the 'shoulders' seen in many other reptile sperm are represented only by a gentle curvature on each side. The length of the nucleus from the base of the acrosome vesicle to the base of the nucleus is 6.6 μm with a further 2.9 μm for the nuclear point which is surrounded by the subacrosomal cone (Fig. 2K). The nucleus is almost parallel sided, showing only a slight increase in width posteriad, from 0.7 to 0.9 μm, reaching its greatest width shortly before its posterior end. The cross section of the nucleus is circular throughout (Figs 2F-J, 3C). The chromatin is condensed and strongly electron dense. Basally the nucleus has a compact conical fossa which houses dense material extending from the proximal centriole (Figs 3A, C, L, 4B, D).

### Neck Region

The neck region (Figs 3A, L, 4B, D) is the region where the nucleus joins the midpiece and is here recognized by virtue of its internal components although the anterior end of the midpiece, as here defined, directly abuts the posterior end of the nucleus. The neck region includes the proximal and distal centrioles and associated densities, including the first of the ring structures of the midpiece. Each centriole consists of 9 triplets. The proximal centriole lies immediately anterior to the distal centriole and with its long axis at slightly less than a right angle to it (Fig. 4B). The long axis of the distal centriole, which forms the basal body of the flagellum, is in the long axis of the axoneme. The centrioles do not lie in the basal nuclear fossa but the proximal centriole, immediately behind this, is surmounted by a hollow conical density (dense cone) which conforms in shape with the nuclear fossa which it occupies. An electron lucent space separates it from the wall of the fossa (Figs 3C, L, 4B, D). Compact dense material extends from the base of the dense cone to cover the more axial end of the proximal

centriole and insinuates itself as a large mass between the proximal and distal centrioles (Figs 3A, L, 4B, D). The two central singlets of the axoneme extend anteriad at least into the region of transition between the distal centriole and the axoneme. In this region there is a density connecting triplet 3 with the adjacent central singlet in addition to the peripheral dense fibre connected to each triplet or doublet (Fig. 3D). The peripheral dense fibre at doublet 3 is detached in some sections, indicating the commencement of the corresponding longitudinal column.

A conspicuous stratified laminar structure forms a wing-like projection on each side of the proximal centriole, near its anterior limit and is continuous around its axial pole (Figs 3A, L, 4B, C, D). It is therefore seen in some longitudinal profiles of the sperm, which are parallel to but not through the long axis of the axoneme, as a continuous wide lamina spanning much of the width of the nucleus behind the nuclear fossa (Fig. 4C). It is deduced, therefore, that the lamina forms a thick disc around the proximal centriole but that the disc is interrupted at the peripheral end of the proximal centriole. Evidence for this interpretation is also seen in some transverse sections of the neck through the proximal centriole (Fig. 4E). The outer edges of the laminar structure make contact with the first of the dense 'ring structures' of the midpiece, described below (Figs 3A, L, 4B, C, D) as does the peripheral end of the proximal centriole (Figs 4B, E).

### Midpiece

The midpiece includes the neck, described above. It consists of mitochondria, ring structures and the contained axoneme with its fibrous sheath and ends posteriorly with the annulus.

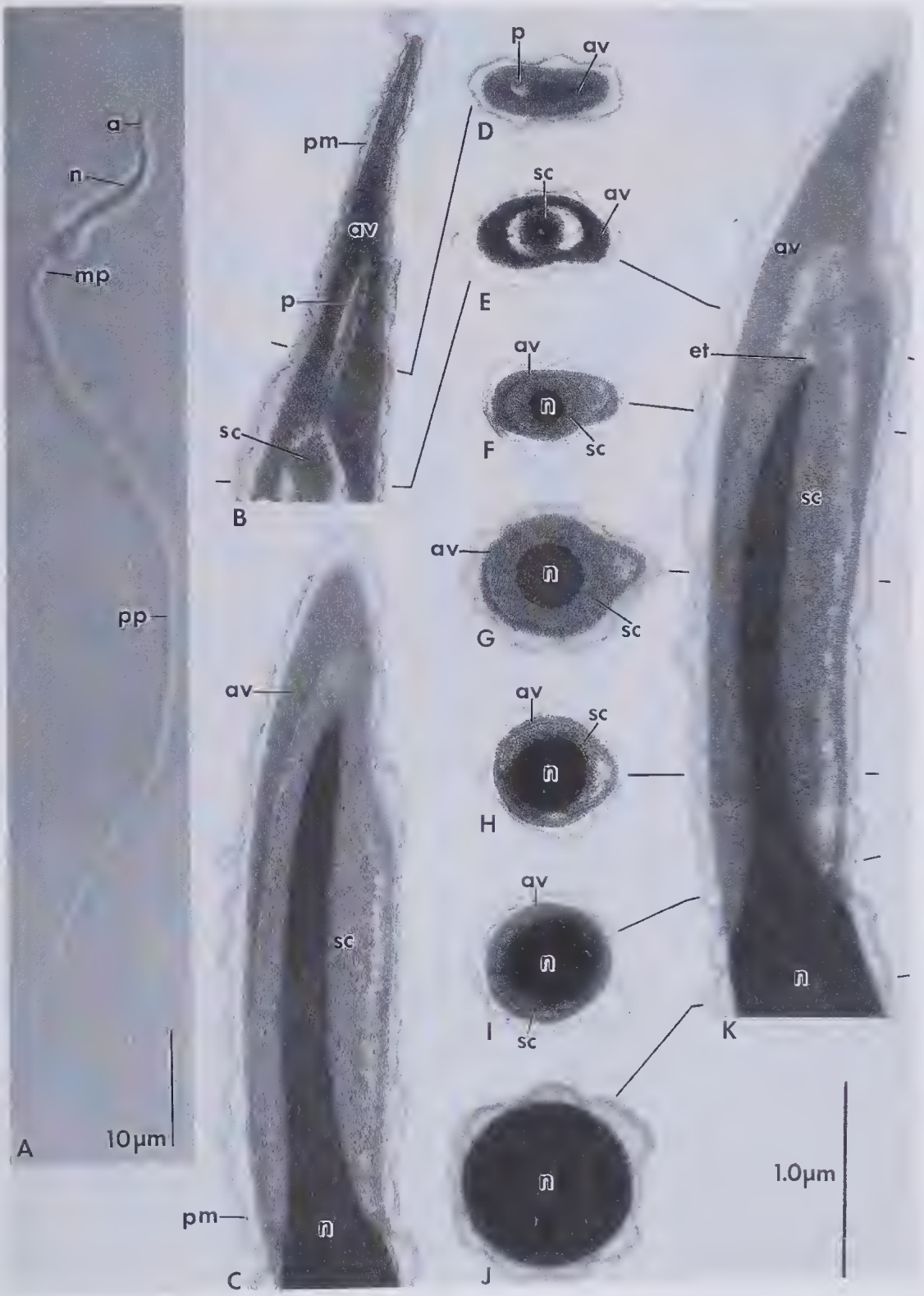
There are four ring structures (rs 1-4) in longitudinal succession, posterior to which lies the much smaller annulus (an). The ring structures, with the annulus, are separated by mitochondrial regions (mi 1-4). In terms of the pattern recognized for the teiid lizard *Cnemidophorus* by Newton and Trauth (1992), the formula for *Nangura* is rs1/mi1, rs2/mi2, rs3/mi3, rs4/mi 4, an. Each ring structure appears in longitudinal section as an approximately kidney-shaped density on each side of the fibrous sheath of the axoneme (Figs 3A, N, 4D). The profile on one side is staggered relative to that on the other, though always overlapping it, but in transverse section the ring is complete, however, when sectioned near its anterior or posterior borders interruption to the ring can be seen (Figs 3E, 4F). This indicates that each structure is a ring which is tilted relative to the axonemal axis.

The mitochondria mostly form elongate, sinuous columnar structures, with numerous predominantly longitudinal cristae, each of which extends from one ring structure to the next (Figs 3A, B, N, 4D). There are 12 or more around the axoneme as seen in transverse section (Fig. 3F). Occasional single, ovoid mitochondria are seen. A few small mitochondrial profiles are sometimes present lateral to the ring structures, the outer surface of which may be scalloped by them (Figs 3E, 4F).

The axoneme has the usual 9+2 pattern. Each doublet has two dynein arms. The A subtubule is occluded by dense material. Around the axoneme almost as far anteriorly as its junction with the distal centriole, there is a fibrous sheath. In longitudinal section (Figs 3A, B, M, N, 4D) the fibrous sheath exhibits rather regularly arranged, approximately square dense blocks which, from glancing longitudinal sections (Figs 3L, N) and transverse sections (Figs 3E-I) are shown to form rings around the axoneme. They show a tendency to tilt relative to the axonemal axis and there are interruptions in the cross sections but that they form a spiral is questionable. Occasional anastomoses of adjacent rings are seen in tangential longitudinal sections.

Nine large peripheral dense fibres are associated with the transition between the distal centriole and the axoneme (Fig. 3D) and continue posteriorly, though much narrower, along the axoneme into the midpiece (Figs 3E, F, 4D). One is attached externally to each triplet or doublet. Within the midpiece, at an undetermined level, all but two of the peripheral fibres become greatly

FIG. 2 *Nangura spinosa*. A.Whole spermatozoon (Nomarski contrast light microscopy). B. Longitudinal section (L.S) through the apical end of the acrosome showing the perforatorium. C. L.S through the basal region of the acrosome showing the nuclear point and paracrystalline matrix of the subacrosomal cone. D-I. A series of transverse sections (T.S) through the acrosome. Note that anteriorly, in D and E, the acrosome is compressed in transverse sections, while further posteriorly, in F-H, it is unilaterally ridged, and at its posterior limit, in I, it is circular. J. T.S through the nucleus. K. L.S through the basal region of the acrosome showing the epinuclear electron lucent region. B-K to the same scale, as indicated. Abbreviations a = acrosome; av = acrosome vesicle; et = epinuclear electron lucent region; mp = midpiece; n = nucleus; p = perforatorium; pm = plasma membrane; pp = principal piece; sc = subacrosomal cone.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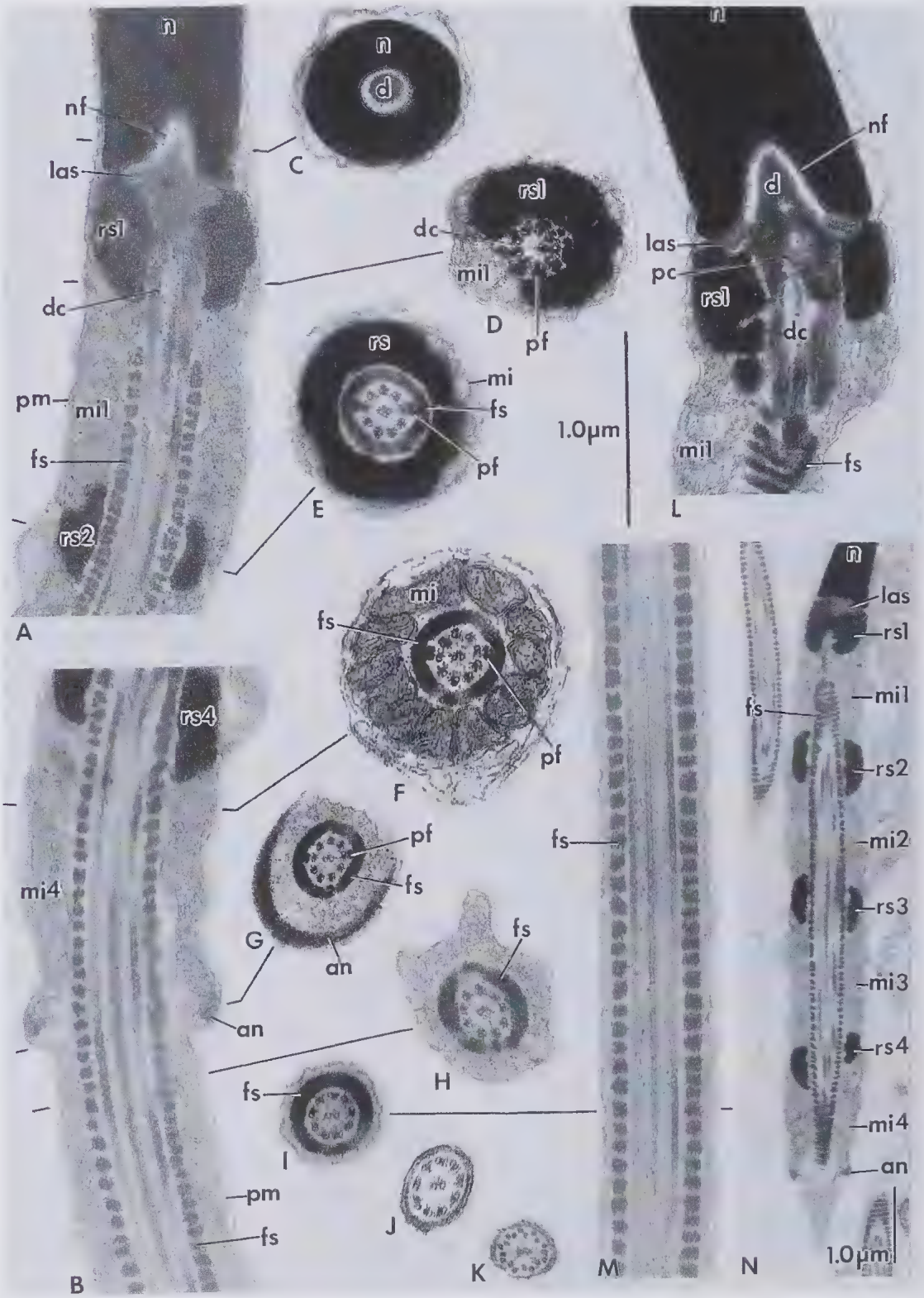
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reduced in size. Only peripheral fibres adjacent to doublets 3 and 8 remain conspieuous, as a double structure nearer the fibrous sheath than it is to its doublet (Figs 3E-G). An unspecified peripheral fibre is seen in longitudinal seetion at the centriolar end of the axoneme to be eross striated (Fig. 4D). The only well developed, though small, peripheral fibres at the level of the annulus are the double fibres at doublets 3 and 8 (dense columns in Fig. 1). At the beginning of the principal piece all nine dense fibres are already vestigial or absent (Figs 3G, H). They are absent from the remainder of the prineipal piece (Fig. 3I).

The annulus (Figs 3B, G, N) is a small dense ring with an irregular oval eross section. It is closely applied to the inner surface of the plasma membrane.

### Principal Piece

The prineipal piece, the longest part of the spermatozoon, eonsists of the continuation, behind the midpiece, of the axoneme with its surrounding fibrous sheath and plasma membrane. It begins, immediately behind the annulus, with a short region in which a wide zone of cytoplasm intervenes between the fibrous sheath and the plasma membrane (Figs 3B, H). The cytoplasm is finely granular, giving the region some resemblanee to a glyeogen pieee but the presence of glycogen has not been determined. Posterior to this the plasma membrane is closely approximated to the fibrous sheath (Figs 3I, M).

### Endpiece

The axoneme projects behind the fibrous sheath as an endpiece of undetermined length (Figs 3J, K).

## DISCUSSION

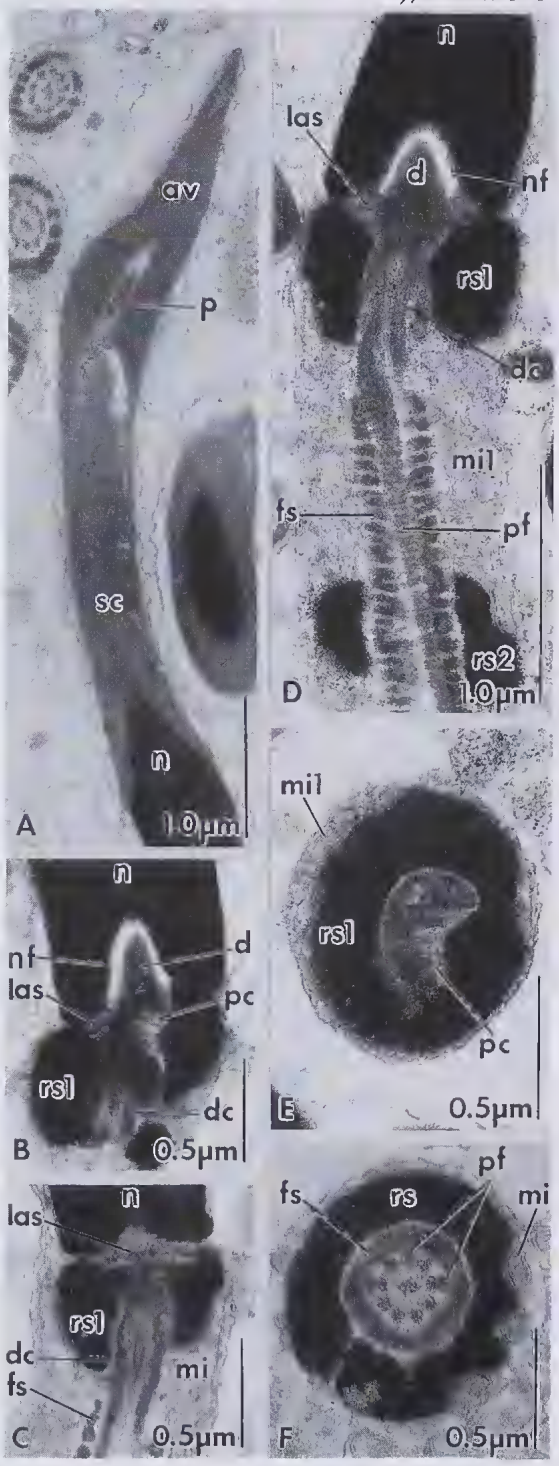
Extension of the fibrous sheath into the midpiece in the sperm of *N. spinosa* is an autapomorphy of the Squamata, unknown in the sperm of other reptiles (Healy & Jamieson, 1992; Jamieson & Healy, 1992) or other amniotes. Newton & Trauth (1992) are incorrect in suggesting that in *Tropidurus* sperm (Da Cruz-Landim & Da Cruz-Hofling, 1977), the fibrous sheath does not extend into the midpiece.

*Nangura* is placed by Covacevich et al. (1993) in the *Sphenomorphus* group of Greer (1979) which also includes *Ctenotus*. Sperm of *Ctenotus robustus* and *C. taeniolatus* have been examined by Jamieson & Scheltinga (in preparation). In the *Eugongylus* group of Greer (1979), Oliver & Jamieson (unpublished data) have examined the sperm of *Cryptoblepharus virgatus*, in the *Eugongylus* subgroup, and *Lampropholis delicata* and *Carlia pectoralis*, in the *Lampropholis* subgroup. The sequence of ring structures in the midpieee of *Nangura* make it more similar to *Ctenotus* than it is to any other examined reptile. Species of the *Eugongylus* group differ in having scattered intermitochondrial bodies (considered homologous with the ring structures) in the form of small dense irregular spheres, tortuous rods or large plates (Oliver & Jamieson, unpublished data). The sperm of *Chalcides ocellatus* examined by Furieri (1970) resemble *Nangura* in having four regularly placed intermitochondrial rings but, unlike *Nangura*, each ring consists of a circlet or small juxtaposed spherules rather than a continuous ring. *Varanus gouldii flavirufus* also has 4 dense intermitoehondrial structures in longitudinal sequenee. However, each 'ring' is made of many loosely aggregated large granules that do not form a continuous ring (Oliver & Jamieson, unpublished data).

In all amniote classes the aerosome plesiomorphically forms an elongate, narrow cone symmetrically located on the tip of the nucleus which it overlaps and constriets. The acrosome vesiele, with this form, encloses a similarly shaped subacrosomal eone the margins of which arc poorly defined in *Nangura*. The pointed form of the aerosome, presenee of the subacrosomal eonc,

FIG. 3 *Nangura spinosa*. A. Longitudinal section (L.S) through the neck region showing, as a squamate autapomorphy, that the fibrous sheath penetrates the midpiece almost to the junction between the axoneme and the distal centriole. B. L.S through the midpiece-principal piece junction showing the annulus. C. Transverse section (T.S) through the nuclear fossa. D. T.S through the distal centriole-axonemal transition showing the 9 peripheral fibres associated with the triplets or doublets. E. T.S through a ring structure. F. T.S through the midpiece showing 12 mitochondria surrounding the axoneme. G. Oblique T.S through the annulus. H and I. T.S through the principal piece. J and K. T.S through the endpiece. L. L.S through the neck region showing the dense cone occupying the conical nuclear fossa. M. L.S through the principal piece. N. L.S through the midpiece showing the four ring structures and annulus separated by four sets of columnar mitochondria. A-M to the same scale, as indicated. Abbreviations an = annulus; d = dense cone; dc = distal centriole; fs = fibrous sheath; las = laminar structure; mi = mitochondria; n = nucleus; nf = nuclear fossa; pc = proximal centriole; pf = peripheral fibre; pm = plasma membrane; rs = ring structure.

and constriction of the nuclear tip are seen in the Chelonia, Crocodilia, *Sphenodon*, Squamata (as in *Nangura*), non-passerine birds (the subacrosomal cone is lost in ratites), and monotremes. These states are also seen in the Lissamphibia, including the primitive frog *Ascaphus* (Jamieson et al., 1993) and presumably characterized the common ancestor of Amphibia and Amniota.




A paracrystalline substructure of the subacrosomal cone, as in *Nangura*, has been recognized in other squamates (Butler & Gabri, 1984; Carcupino et al., 1989; Furieri, 1970), including *Ctenotus* (Jamieson & Scheltinga, in preparation). It is probably a synapomorphy, and autapomorphy, of the Squamata.

All classes of amniotes possess one or more endonuclear canals, containing one or more perforatoria, which penetrate the anterior end of the nucleus to varying depths. This condition is also seen in basal lissamphibians: urodeles (Picheral, 1967) and primitive anurans (see Jamieson et al., 1993; Sandoz, 1970). There are also endonuclear canals in *Latimeria*, and *Neoceratodus* (see Jamieson, 1991), therefore the presumed common ancestor of Amphibia and amniotes probably possessed one or more endonuclear canals. Absence of endonuclear canals is a synapomorphy of the squamates and a homoplasy of these with nonpasserines and monotremes (Jamieson & Healy, 1992). However, squamates, including *Nangura*, retain a perforatorium anterior to the nucleus.

The nucleus is an elongate cylinder narrowly constricted within the base of the acrosome in some members of all amniote classes (Jamieson & Healy, 1992). This form, also seen in *Ascaphus* and urodeles (Jamieson et al., 1993), is clearly plesiomorphic for amniotes. It is widespread in reptiles, including *Nangura*.

Representation of the basal nuclear fossa is variable in amniotes. It is poorly developed in the sperm of the caiman, and is small and compact in turtles, *Sphenodon* (tuatara), rooster, guinea fowl, and squamates excepting the skinks. In skinks it

FIG. 4 *Nangura spinosa*. A. Longitudinal section (L.S) through the full length of the acrosome. B-D. L.S through the neck region showing, B, the oblique angle of the proximal centriole relative to the distal centriole, C, the laminar structure, and D, a cross striated peripheral fibre. E. T.S through the proximal centriole showing that the microtubules of the proximal centriole make contact with the ring structure. F. T.S through a ring structure showing the outer surface scalloped by small mitochondria. Abbreviations av = acrosome vesicle; d = dense cone; dc = distal centriole; fs = fibrous sheath; las = laminar structure; mi = mitochondria; n = nucleus; nf = nuclear fossa; p = perforatorium; pc = proximal centriole; pf = peripheral fibre; rs = ring structure; sc = subacrosomal cone.

is narrowly funnel-shaped or conical, as in *Nangura*. In the ratites it has a triple profile. The small and compact form may be plesiomorphic for amniotes (Jamieson & Healy, 1992).

A dense ring, the annulus, at the posterior end of the midpiece is a feature of many metazoan sperm and is clearly plesiomorphic for amniotes. It has been demonstrated, inter alia, in turtles, crocodile, tinamou, rhea, rooster, guineafowl, tuatara and monotremes. Squamates were considered exceptional in absence or at most negligible development of an annulus (Jamieson & Healy, 1992). However, an annulus has been demonstrated for *Lacerta vivipara* by Courtens & Depeiges (1985), *Cnemidophorus sexlineatus* by Newton & Trauth (1992) and for *Nangura*, and may be more widely present in squamates than previously suspected.

The number of mitochondria seen in transverse section of the midpiece, where possible near its anterior end, is very variable in amniotes. The number in chelonians, here considered the most basal amniotes, is six. A trend towards reduction to four in birds and monotremes has been observed. There are eight or nine in the tuatara, the caiman and the skink *Chalcides ocellatus* (Jamieson & Healy, 1992). In the teiid *Cnemidophorus sexlineatus* there are 8 to 10 (Newton & Trauth, 1992). In snakes, the number shows apomorphic increase to as many as 14. The number, approximately 12 in *Nangura*, is also apomorphically high.

The predominantly linear arrangement of cristae in the mitochondria of *Nangura* sperm is a usual feature of amniotes, including most reptiles. Only turtles, caiman and the tuatara, are known to be exceptional. The mitochondrial cristae in these three taxa are concentric and usually surround a large central dense body. In all other amniotes, excepting the Wooly opossum which also has concentric cristae, the cristae have a 'conventional' appearance, being linear or curved but never concentric, and do not surround a dense body. The concentric arrangement around a dense body is here considered to be an apomorphy acquired early in amniote evolution, as evidenced by its occurrence in Chelonia but later lost (Jamieson & Healy, 1992).

The intermitochondrial rings, 'ring structures' in *Nangura*, which are limited to squamates are regarded as derivations of the dense bodies of basic amniotes (Jamieson & Healy, 1992). Carcupino et al. (1989), independently concluded that the rings in *Chalcides ocellatus tiligugu*, were mitochondrial derivatives. Origin of intermitochondrial material from mitochondria has been confirmed by demonstration ontogenetically in the sperm of some squamates by Oliver & Jamieson (in preparation).

The distal centriole, forming the basal body of the axoneme, is plesiomorphically short in vertebrates, including the Lissamphibia, as in most Metazoa, and in *Nangura*. In contrast, the distal centriole extends the entire length of the long midpiece in the tuatara, turtles, crocodiles, and ratites, an apparent basal synapomorphy of amniotes. The shorter, though still elongate distal centriole in the rooster and the somewhat shorter centriole in guinea fowl, the short centriole in squamates, and the vestigial, possibly absent, centriole in monotremes possibly represent secondary reduction in length of the centriole (Healy & Jamieson, 1992).

A cross striated dense body lateral to the proximal centriole, represented by the laminated structure in *Nangura*, appears to be a basal synapomorphy of amniotes but its homology across the various groups requires confirmation. It is seen in tuatara, and the caiman where homology with the striated columns of eutherian sperm has been suggested (Healy & Jamieson, 1992). It has not been reported for sperm of birds or monotremes and the squamates but in view of its presence in *Nangura* further examination of squamates is needed.

The annulated, dense fibrous sheath seen in *Nangura* must have developed in the earliest amniotes as it is present in all amniote classes (though it is absent in some birds). Occasional anastomoses of adjacent rings are seen in tangential longitudinal sections and similar 'branching' in the snake *Lampropeltis getulus*, led Austin (1965) to propose that the annuli are linked together along one or both sides of the tail. A fibrous sheath is absent from amphibian sperm.

The isolated peripheral fibres 3 and 8 in amniotes may well be homologous with columns at this point in other sarcopterygians, the coelacanth, *Latimeria*, and Dipnoi. However, such modifications at doublets 3 and 8, which are approximately in the plane of the two central singlets, could be independent acquisitions as they presumably are in chondrichthyan sperm (references in Jamieson, 1991). In contrast with reptiles, in eutherian mammals the outer coarse fibres at 3 and 8 are the smallest and they terminate first, their place being occupied throughout most of the length of the principal piece by inward prolongations of the dorsal and

ventral portions of the fibrous sheath (Hamilton & Fawcett, 1968).

Nine longitudinal dense fibres peripheral to the 9 axonemal doublets, as in *Nangura*, are a fundamental feature of amniote sperm, being found in all classes. They are an autapomorphy and simultaneous symplesiomorphy of the amniotes. The peripheral dense fibres are small in most amniotes investigated: turtles, the caiman, tuatara, squamates and monotremes. The peripheral fibres are described as 'tiny' for the rhea. They are present in suboscine and the more apomorphic oscine passerines, being larger in the latter. They are large and diverse in shape in marsupials above the didelphids, and in eutherian mammals. There thus appear to be trends to enlargement of the peripheral fibres in passerines and non-monotreme mammals, with diversification in the latter.

The peripheral fibres are usually situated in the midpiece with some extension into the principal piece as in turtles, the caiman, non-passerines, tuatara, and monotremes. In eutherians and marsupials they extend far into the principal piece. However, in *Nangura*, as in other squamates, the only well developed, though small, peripheral fibres at the level of the annulus are the double fibres at doublets 3 and 8 and by the beginning of the principal piece all nine dense fibres are already vestigial or absent. In non-passerine birds the fibres may be restricted either to the midpiece or to the principal piece or occur in both or, as in doves, are absent (Jamieson & Healy, 1992; Asa & Phillips, 1987; Jamieson, unpublished data).

In turtles, the tuatara and the skinks *Nangura* and *Chalcides* see Furieri (1970), the nine peripheral dense fibres are partly displaced from the radii of the doublets into the gaps between adjacent doublets. Their exact radial position is unknown in the crocodile and in most squamates. In contrast, the peripheral dense fibres lie in the same radius as the doublets in the rhea, at least some non-passerines (turkey) and monotremes (Jamieson & Healy, 1992).

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